Potential Antitumor Agents. 56. "Minimal" DNA-Intercalating Ligands as Antitumor Drugs: Phenylquinoline-8-carboxamides

Graham J. Atwell, Claudia D. Bos, Bruce C. Baguley, and William A. Denny*

Cancer Research Laboratory, University of Auckland School of Medicine, Private Bag, Auckland, New Zealand.
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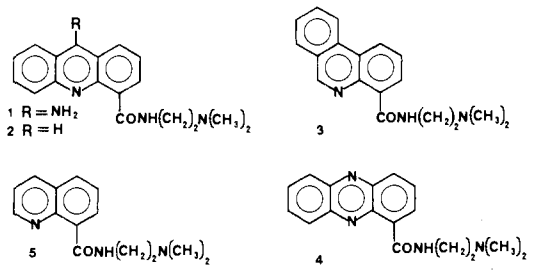
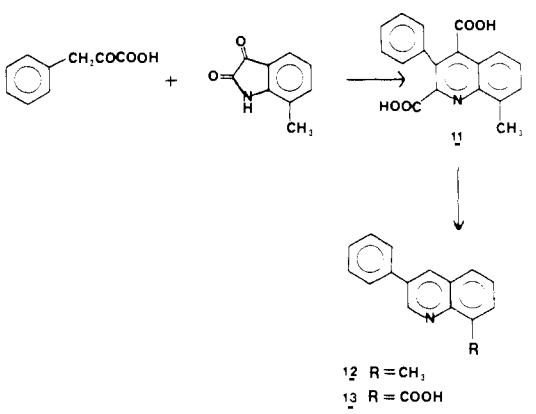
A series of isomeric phenylquinoline-8-carboxamides have been synthesized and evaluated as antitumor agents. This configuration is close to the minimum chromophore required for intercalative binding, since the binding mode of the compounds is dependent on the presence and position of the phenyl ring. If the ring is appended at the 4- or 5-position, it cannot lie within the DNA-intercalation site, and the compounds do not intercalate as shown by both unwinding and helix extension assays. In contrast, the 2-, 3-, and 6-phenyl isomers (where the phenyl ring lies coplanar with the quinoline and in the intercalation site) bind by intercalation. Only those isomers that intercalate show in vivo antitumor activity, with the 2-phenyl derivative in particular possessing broad-spectrum activity in both leukemia and solid-tumor assays.

Much recent work on the development of DNA-intercalating agents as antitumor drugs has concentrated on structures capable of in vivo activity against remotely sited solid tumors.[1-5] An essential requirement for such broad-spectrum activity is the ability of the drug to distribute from the point of administration to remote tumor sites. While overall drug lipophilicity is important in this regard, the provision of an uncharged intercalating chromophore appears critical. For example, the 9-aminoacridine-4-carboxamide **1** (acridine $pK_a = 8.30$) is a highly active antileukemic agent but shows no activity against the remotely sited Lewis lung carcinoma, whereas the more weakly basic acridine-4-carboxamide **2** (acridine $pK_a = 3.54$) shows good activity in both tumor models.[3] Consonant with this finding, other weakly basic tricyclic compounds such as the phenazines (**3**) and phenanthridines (**4**) also show broad-spectrum activity.[6,7]



Another property governing drug distribution is likely to be the strength of DNA binding. Various quantitative structure–activity relationship (QSAR) studies have demonstrated a positive relationship between the DNA binding strength of intercalating agents and biological potency, in both in vitro[8] and in vivo[9-11] leukemia models. However, this relationship does not hold for activity against remotely implanted solid tumors,[12] where drug distribution properties may be much more important, and in fact there may be merit in developing compounds of minimum DNA binding strength, provided the intercalative binding mode is maintained. Although a high proportion of drug will remain DNA bound, the corresponding significant increase in the amount of free drug at equilibrium may have important effects on distribution.

Scheme I



One way of lowering DNA binding strength is to reduce the size of the chromophore. This has the additional advantage that "nonspecific" binding to other cell macromolecules such as proteins and lipids (which correlates with chromophore size and lipophilicity[13]) will also be reduced. We have thus been interested in defining the minimum chromophore requirements for intercalative binding.[14] While there is a general concensus that three fused aromatic rings are required for efficient intercalative binding, even one-ring chromophores of appropriate electron deficiency[15] have been reported to intercalate DNA.

For this particular study, we chose to focus on the quinolinecarboxamide derivative **5**, which is known to bind to DNA in a nonintercalative fashion (Table I and ref 14) and is inactive as an antitumor agent. Addition of a third fused benzene ring to give either **2** or **3** ensures intercalative binding and broad-spectrum biological activity.[3,6] In this paper we look at the consequences of appending a

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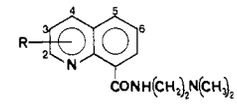
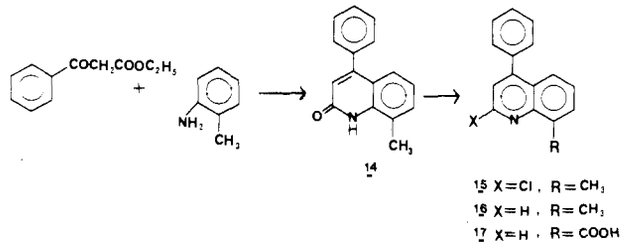
Table I. Physiochemical and Biological Data for Substituted Quinoline-8-carboxamides



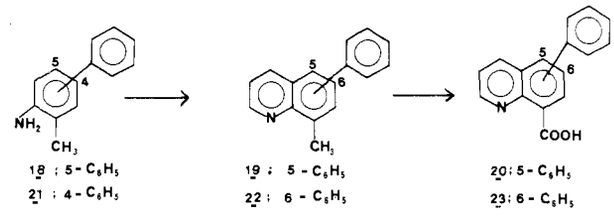
						DNA binding: cytotoxicity, in vivo activity						
						log K^e			P388		LL	
no.	R	R_m[a]	pK_a[b]	ϕ[c]	slope[d]	AT	GC	IC_{50}[f]	OD[g]	ILS_{max}[h]	OD	ILS_{max}
2		−0.02	3.54	16		6.12	6.54	105	66	91	100	− (6)[k]
5	H	−0.38	3.07	0	0.0	5.12	5.17	>25000	100	NA[i]		NT[j]
6	2-Ph	−0.01	2.75	14	0.6	5.97	5.92	1300	100	91	100	70 (4)
7	3-Ph	0.03	2.58	20	1.55	5.68	5.70	2600	100	27	100	NA
8	4-Ph	0.03	3.04	0	0.2	4.73	4.77	14700	150	NA		NT
9	5-Ph	0.15	3.13	0	0.17	4.74	4.79	13700	100	NA		NT
10	6-Ph	0.04	2.93	20	0.9	6.12	5.96	910	65	29	100	31

[a] Chromatographic measure of compound lipophilicity determined by liquid–liquid thin-layer chromatography on a cellulose support (ref 21). [b] Ionization constant of chromophore measured by UV spectroscopy in 20% dimethylformamide (ref 32). [c] Unwinding angle (degrees) shown by the compound when bound to cloned circular supercoiled DNA from the plasmid pNZ116, relative to ethidium as 26° (ref 23, 33). [d] Slope of the plot of increase in viscosity of a solution of short rodlike DNA versus increasing D/P ratio (ref 23, 24). [e] Association constants (M^{-1}) for binding to poly[d(AT)] and poly[d(GT)], determined by the ethidium displacement method (ref 22). [f] Drug concentration (nM) that reduces cell growth of L1210 leukemia cultures to 50% of controls after 70 h. Figures are the average of at least three independent determinations and the percentage standard error is ≤7%. [g] Optimal drug dose (mg/kg) administered intraperitoneally in 0.1 mL of ethanol/water (30% v/v), on days 1, 5, and 9 after intraperitoneal inoculation (on day 0) of 10^6 P388 leukemia cells or on days 5, 9, and 13 after intravenous inoculation (on day 0) of 10^6 Lewis lung carcinoma cells. Compounds were tested at 1.5× dose intervals over the range from inactive to acutely toxic. [h] Percentage increase in lifespan of treated animals (at the optimal dose) compared to control animals. Figures are the mean of two determinations. Average lifespan of control animals was 11 days (P388) or 17 days (Lewis lung). [i] Compound inactive (ILS < 20% for P388, < 40% for Lewis lung) at all doses up to toxic ones. [j] Compound not tested in this system. [k] Values in parentheses are the number of animals (out of a group of six) that were long-term survivors (held 50 days for P388, 60 days for Lewis lung).

Scheme II



Scheme III



nonfused benzene ring to **5** in various ways, to give the phenylquinoline derivatives **6–10** listed in Table I.

Chemistry

Acquisition of the compounds listed in Table I required the synthesis of the isomeric phenylquinoline-8-carboxylic acids. On a small scale, these were most conveniently obtained by controlled high-temperature (200–300 °C) oxidation of the corresponding methyl intermediates with SeO_2. 2-Phenylquinoline-8-carboxylic acid was prepared by literature methods.[16] A Doebner pyruvic acid synthesis[17] gave 8-methyl-2-phenylquinoline-4-carboxylic acid, which was decarboxylated and then oxidized with SeO_2. This method was preferred to the higher yielding Pfitzinger method because of its simplicity and the low cost of starting materials. 3-Phenylquinoline-8-carboxylic acid (**13**) was prepared by the method outlined in Scheme I. Reaction of phenylpyruvic acid with 7-methylisatin gave the quinoline diacid **11**, which on decarboxylation and SeO_2 oxidation gave the desired compound **13** in 22% overall yield from **11**.

4-Phenylquinoline-8-carboxylic acid (**17**) was prepared by the method outlined in Scheme II. Condensation of ethyl benzoylacetate and 2-methylaniline following the procedure of Hauser and Reynolds[18] gave the 4-phenylquinolone **14**, which was converted to the 2-chloroquinoline **15**, dechlorinated to give 8-methyl-4-phenylquinoline (**16**), and then oxidized to the acid **17**. Although 8-methyl-4-phenylquinoline has been prepared by pyrolysis of cinnamaldehyde 2-methylanil,[19] the yield by this route was low, and the method did not seem practicable. 4-Phenylquinoline itself has been prepared by the condensation of 3-bromoprophenone and aniline,[20] but when this method was attempted with 2-methylaniline, the yields were found to be unacceptably low.

The 5- and 6-phenylquinoline-8-carboxylic acids (**20** and **23**) were obtained by the general method outlined in Scheme III. Use of the appropriate aminomethylbiphenyl (**18, 21**) in the Skraup synthesis gave the corresponding phenyl-8-methylquinolines (**19** and **22**), which were oxidized with SeO_2.

Results and Discussion

A phenyl ring can be attached to the parent quinoline-8-carboxamide **5** at six different positions. Severe problems were encountered in the attempted synthesis of the 7-phenyl isomer, presumably due to steric effects between the 7-phenyl and 8-carboxy groups, and its preparation was not pursued. Previous studies with substituted acridine-4-carboxamides show that, when the side chain is attached ortho to a chromophore substituent, the resultant (assumed) steric effects render the compound atypical in terms of both DNA binding and biological activity.[3] The

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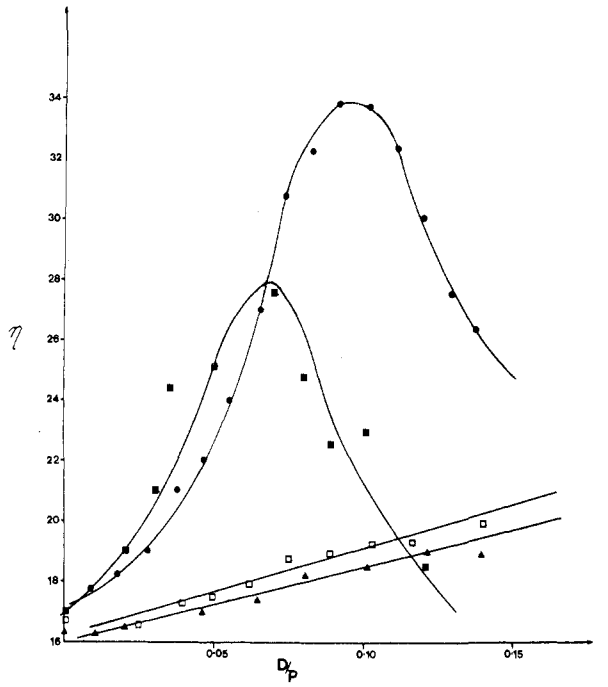

Figure 1. Effect of selected compounds of Table I on the reduced viscosity of covalently closed circular DNA. The ordinate represents the reduced viscosity in dL/g, and the abscissa shows the molar binding ratio (drug molecules/DNA phosphate). The equivalence binding ratio for ethidium (r_e) is 0.055 (data not shown), while those for compounds **2** (●) and **7** (■) are 0.09 and 0.07, respectively. Unwinding angles relative to ethidium were calculated from the formula $\phi_d = 26r_e/r_d$ as 16° and 20°, respectively. Compounds **5** (▲) and **9** (□) did not unwind or rewind the DNA.

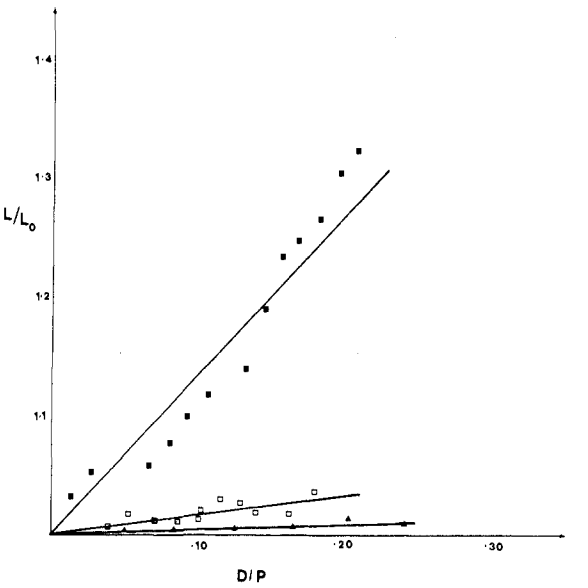

Figure 2. Effect of selected compounds of Table I on the relative contour length of sonicated calf thymus DNA. The ordinate represents the calculated ratio of contour lengths in the presence (L) or absence (L_0) of the drugs. The abscissa shows the molar binding ratio (drug molecules/DNA phosphate), and the lines are the least-squares fit. Compound **7** (■), compound **5** (▲), compound **9** (□).

other five compounds in the series were prepared, and physicochemical and biological data for these are recorded in Table I, together with comparative data for the parent quinoline-8-carboxamide **5** and the acridine-4-carboxamide **2**, which has the additional benzene ring fused rather than appended to the chromophore.

The overall lipophilicities of the compounds were determined by liquid–liquid thin-layer chromatography[21] and are as expected. Only a small increase in lipophilicity is seen when the benzene ring is fused to **5** to give **2**, but when appended to give **6–10**, the lipophilic effect is more marked. Addition of the phenyl ring at positions 4 and 5 (compounds **8** and **9**) had virtually no effect on the pK_a of the quinoline, whereas in positions 2, 3, and 6 it has an appreciable base-weakening effect. However, in all cases the chromophore pK_a is sufficiently low that the compound will exist under physiological conditions as the monocation, with the chromophore uncharged.[3]

The quinoline-8-carboxamide **5** binds moderately well to DNA, as determined by the ethidium displacement assay[22] (Table I), but has been shown previously[14] not to unwind closed circular supercoiled DNA (Figure 1). The nonintercalative binding mode of **5** was further confirmed by the zero slope this compound showed in the helix extension assay,[23] indicating its inability to extend the contour length of short, rodlike DNA (Figure 2).

Fusion of a benzene ring gives the acridine-4-carboxamide **2**, which binds to DNA about 10-fold more strongly as determined by association constants (Table I). This compound has been shown[3] to intercalate DNA with an unwinding angle of 12° (relative to ethidium at 26°).

When the phenyl ring is appended at position 2, 3, or 6 (i.e., off the long axis) of the quinoline-8-carboxamide, the resultant phenylquinolines (**6**, **7**, and **10**) all show evidence of intercalative binding, unwinding, and rewinding DNA with unwinding angles from 15° to 20° (Table I and Figure 1). They also show positive slopes of from 0.6 to 1.6 in the helix extension assay (Figure 2). While the value of 0.6 shown by the 2-phenyl derivative **6** is at the lower limit of acceptability for intercalative binding,[23,24] it is nevertheless distinctly different from the essentially zero value shown by the parent quinoline-8-carboxamide **5**. The combined results of both assays clearly establish that the phenylquinolines (**6**, **7**, and **10**) bind to DNA by intercalation.

In striking contrast, phenyl groups appended at positions 4 and 5 (off the short axis of the chromophore) provided compounds **8** and **9**, which show no evidence of intercalative binding in either assay. They do not appear to unwind closed circular supercoiled DNA (Figure 1) and show slopes in the helix extension assay of essentially zero (Figure 2). They also show significantly lower association constants for binding to DNA compared to the other phenylquinoline isomers, as determined by the ethidium displacement assay (Table I). Although the absolute log K values calculated for these compounds may not be strictly comparable to those for the intercalating isomers, since the assay assumes competitive binding of ethidium and the ligand for intercalation sites, there is no doubt that **8** and **9** have an affinity for DNA about 1 order of magnitude less than those of the intercalating isomers.

Molecular models show that, if the carboxamide side chains of these compounds reside in the DNA minor groove as suggested for the 9-aminoacridine-4-carboxamides,[25] the

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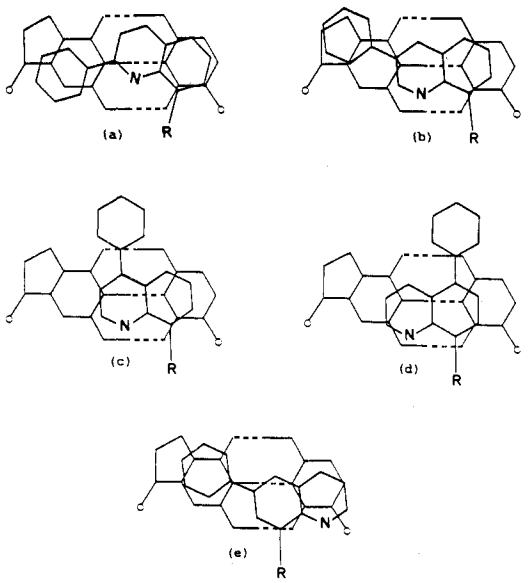

Figure 3. Most probable binding orientations for phenylquinolinecarboxamide isomers, deduced from CPK models. All complexes show the base pair on top, with the drug carboxamide side chain R protruding up. (a) 2-Ph, (b) 3-Ph, (c) 4-Ph, (d) 5-Ph, (e) 6-Ph.

quinoline chromophore will be oriented in such a way that the phenyl rings of the 2-, 3-, and 6-phenylquinoline-8-carboxamides will also reside in the intercalation site, contributing to the intercalative binding energy (Figure 3). Preliminary crystallographic studies on the 2-phenyl isomer **6** indicates the phenyl ring lies at an angle of only 20° to the quinoline,[26] and the nonbonded interactions between the ortho hydrogens are likely to be sufficiently low that the compounds can assume essentially coplanar conformations at little energy cost.

In contrast, phenyl rings attached at positions 4 and 5 (off the short axis) in compounds **8** and **9** will not lie in the intercalation site but will protrude into the major groove (Figure 3). Additionally, the more severe nonbonded interactions between the phenyl ortho hydrogens and the peri hydrogens of the quinoline makes it much less likely that compounds **8** and **9** can assume coplanar conformations (see ref 27 for an analogy).

The parent nonintercalating quinoline-8-carboxamide **5** shows very low cytotoxic potency, with an IC_{50} of greater than 25 000 nM, compared to the DNA-intercalating acridine-4-carboxamide with an IC_{50} of 105 nM. The three intercalating phenylquinolines show reasonable levels of potency (1300–2600 nM) and are much more potent than the isomeric nonintercalating derivatives. The parent quinoline-8-carboxamide **5** and the two nonintercalating phenylquinolines **8** and **9** also show no in vivo activity against the P388 leukemia. However, all three of the intercalating isomers (**6**, **7**, and **10**) have confirmed in vivo activity. While that of compounds **7** and **10** is of low order and does not extend to Lewis lung activity, the 2-phenylquinoline-8-carboxamide **6** shows broad-spectrum in vivo activity comparable to that of the acridine-4-carboxamide **2**, with a high proportion of long-term survivors in the Lewis lung solid-tumor assay.

Conclusions

It is generally accepted that three fused aromatic rings in a chromophore is the minimum requirement to ensure intercalative binding to DNA. In the present case, where the third phenyl ring is appended but not fused to the chromophore, the mode of binding is variable and dependent upon the positioning of the phenyl ring. When the appended ring can accomodate itself in a virtually coplanar fashion within the DNA binding site, intercalative binding is favored, but if this cannot occur (as is probable with compounds **8** and **9**), then binding is nonintercalative.

The results also clearly show again the necessity for intercalative binding for activity among compounds of this broad class. The three intercalating isomers showed potent in vitro cytotoxicity and were active in vivo, while the two nonintercalating isomers were not.

More importantly, the results also indicate that provided this criterion is met, molecules with broad-spectrum antitumor activity are not limited to those possessing fused tricyclic or larger chromophores. The biological activity of the 2-phenylquinoline-8-carboxamide **6** is very encouraging, and further development of this class of "minimal" DNA-intercalating ligands is being carried out.

Experimental Section

Where elemental analyses are indicated only by the symbols of the elements, results obtained were within ±0.4% of the theoretical values. Analyses were carried out in the Microchemical Laboratory, University of Otago, Dunedin. Melting points were determined on an Electrothermal apparatus, with use of the supplied stem-corrected thermometer, and are as read.

2-Phenylquinoline-8-carboxylic Acid. 8-Methyl-2-phenylquinoline was prepared by literature methods[17] and oxidized with chromic anhydride to give the acid, mp 159–160 °C (lit.[16] mp 158–159 °C).

3-Phenylquinoline-8-carboxylic Acid (13). A mixture of phenylpyruvic acid (26 g, 158 mmol) and 7-methylisatin (24.2 g, 151 mmol) in 15% aqueous NaOH (150 mL) was heated under gentle reflux for 3 h. The cooled solution was treated with charcoal, acidified with 2 N HCl, and cooled at 5 °C for 10 h. The precipitate was collected and extracted with 2 N $NaHCO_3$. The aqueous layer was extracted with EtOAc and then acidified with 2 N HCl to give crude 8-methyl-3-phenylquinoline-2,4-dicarboxylic acid (**11**) (44.7 g, 96%), suitable for the next step. Crystallization of a sample from MeOH/EtOAc gave needles, mp 181–183 °C. Anal. ($C_{18}H_{13}NO_4$) C, H, N.

The above diacid was heated with Cu powder (3 g) at 270–280 °C for 15 min. The cooled reaction mixture was extracted with petroleum ether to give 8-methyl-3-phenylquinoline (**12**) (yield 77%) of sufficient purity for oxidation. A sample was crystallized from MeOH/EtOAc/HCl as the hydrochloride, mp 177–179 °C. Anal. ($C_{16}H_{13}N$·HCl) C, H, N, Cl.

A finely ground mixture of the above free base (5.0 g, 23 mmol) and SeO_2 (5.5 g, 50 mmol) was heated to 180–190 °C when a vigorous exothermic reaction occurred, raising the temperature to 270–290 °C. The mixture was then held at 280 °C for 2 min, cooled, and extracted three times with boiling $CHCl_3$. The combined extracts were evaporated, and the residue was extracted with hot 2 N aqueous KOH. The extract was clarified with charcoal, partially neutralized with AcOH, and filtered to remove impurities. Further addition of AcOH then precipitated the crude product. Several crystallizations from EtOH gave pure 3-phenylquinoline-8-carboxylic acid (**13**) as white prisms (1.64 g, 29%), mp 133–135 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

4-Phenylquinoline-8-carboxylic Acid (17). 8-Methyl-4-phenyl-2(1*H*)-quinolone[16] (**14**) was refluxed in $POCl_3$ to provide 2-chloro-8-methyl-4-phenylquinoline (**15**), which was crystallized from MeOH as colorless needles (81% yield), mp 48–48.5 °C. Anal. ($C_{16}H_{12}ClN$) C, H, N, Cl. This chloroquinoline (7.3 g, 29 mmol) was dissolved in a mixture of petroleum ether/MeOH containing Et_3N (4.8 g, 1.2 equiv) and hydrogenated (Pd/C at 60 psi) for 12 h. The product was separated from small amounts of starting material by formation and crystallization of the picrate salt, mp 214–215 °C (lit.[19] mp 217 °C). Regeneration then gave pure 8-methyl-4-phenylquinoline (**16**) (4.8 g, 76%) as an oil.

This was oxidized by the SeO_2 method. Crystallization of the product from CH_2Cl_2/petroleum ether and then from aqueous

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Table II. Physical Properties for the New Compounds of Table I

no.	mp, °C	formula	anal.
6	118–120	$C_{20}H_{21}N_3O \cdot 2HCl$	C, H, N, Cl
7	118–120	$C_{20}H_{21}N_3O \cdot 2HCl$	C, H, N, Cl
8	132–134	$C_{20}H_{21}N_3O \cdot HCl \cdot H_2O$	C, H, N
9	174–175	$C_{20}H_{21}N_3O \cdot HCl$	C, H, N, Cl
10	199–200	$C_{20}H_{21}N_3O \cdot 2HCl$	C, H, N, Cl

MeOH gave the desired 4-phenylquinoline-8-carboxylic acid (**17**) (31% yield), mp 161–161.5 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

5-Phenylquinoline-8-carboxylic Acid (20). A mixture of 3-amino-4-methylbiphenyl[28] (**18**) (11.5 g, 63 mmol), glycerol (21.5 g), anhydrous As_2O_5 (11 g), and concentrated H_2SO_4 (19.5 g) was stirred at 160–165 °C for 5 h, as described for the general Skraup synthesis of pyridylquinolines.[29] The cooled mixture was diluted with aqueous HCl, clarified with charcoal, filtered, and extracted with CH_2Cl_2. The residue of this extraction was then extracted with boiling petroleum ether to give 8-methyl-5-phenylquinoline (**19**) (8.8 g, 64%). A sample crystallized from aqueous MeOH as colorless prisms, mp 57.5–58 °C. Anal. ($C_{16}H_{13}N$) C, H, N.

SeO_2 oxidation of this compound by the above procedure gave 5-phenylquinoline-8-carboxylic acid (**20**). Crystallization from CH_2Cl_2/EtOH then EtOAc gave pure product (34% yield), mp 150–151 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

6-Phenylquinoline-8-carboxylic Acid (23). 3-Methyl-4-nitrobiphenyl[30] was reduced by Fe/HCl in DMF/EtOH/H_2O, and the resulting 4-amino-3-methylbiphenyl (**21**) was purified by crystallization of the hydrochloride salt from EtOAc/MeOH/HCl as prisms, mp 232–235 °C. Anal. ($C_{13}H_{13}N \cdot HCl$) C, H, N. This was used in the Skraup synthesis under identical conditions with those described above and provided 8-methyl-6-phenylquinoline (**22**) in 78% yield. A sample was crystallized from EtOAc/Me_2CO/HCl as the hydrochloride salt, mp 263–265 °C. Anal. ($C_{16}H_{13}N \cdot HCl$) C, H, N, Cl.

Oxidation with SeO_2 as described above, followed by crystallization of the product from EtOH and then EtOAc, gave 6-phenylquinoline-8-carboxylic acid (**23**) (31% yield), mp 170–171 °C. Anal. ($C_{16}H_{11}NO_2$) C, H, N.

Synthesis of Amides of Table I. General Example. 2-Phenylquinoline-8-carboxylic acid (1 equiv) was suspended in dry DMF (5 mL/g) and treated with 1,1′-carbonyldiimidazole[31] (1.5 equiv) at 20–50 °C for 30 min (until gas evolution ceased). The mixture was cooled to 20 °C and treated with *N*,*N*-dimethylethylenediamine (2.5 equiv). After 15 min, most of the solvent was removed under reduced pressure, and dilute aqueous Na_2CO_3 was added to precipitate the product. Extraction with CH_2Cl_2 gave the crude free base, which was crystallized from MeOH/EtOAc/HCl to give a 90% yield of *N*-[2-(dimethylamino)ethyl]-2-phenylquinoline-8-carboxamide dihydrochloride (compound **6** of Table I), mp 118–120 °C. Anal. (Table II). The other compounds of Table I were prepared similarly.

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Registry No. **2**, 89459-25-6; **5**, 112022-03-4; **6**, 107027-12-3; **6**·2HCl, 107026-86-8; **7**, 113431-33-7; **7**·2HCl, 113431-34-8; **8**, 113431-35-9; **8**·HCl, 113431-36-0; **9**, 113431-37-1; **9**·HCl, 113431-38-2; **10**, 113431-39-3; **10**·2HCl, 113431-40-6; **11**, 113431-41-7; **12**, 113431-42-8; **12**·HCl, 113431-43-9; **13**, 113431-44-0; **14**, 70453-86-0; **15**, 113431-45-1; **16**, 113431-46-2; **17**, 113431-47-3; **18**, 80938-67-6; **19**, 113431-48-4; **20**, 113431-49-5; **21**, 63019-98-7; **21**·HCl, 3419-49-6; **22**, 113431-50-8; **22**·HCl, 113431-51-9; **23**, 113431-52-0; $PhCH_2COCO_2H$, 156-06-9; *o*-$MeC_6H_4NH_2$, 95-53-4; $PhCOCH_2CO_2Et$, 94-02-0; 8-methyl-2-phenylquinoline, 5353-90-2; 2-phenyl-8-quinolinecarboxylic acid, 5093-81-2; 7-methylisatin, 1127-59-9; glycerol, 56-81-5; 3-methyl-4-nitrobiphenyl, 69314-47-2; *N*,*N*-dimethylethylenediamine, 110-70-3.

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(Acyloxy)benzophenones and (Acyloxy)-4-pyrones. A New Class of Inhibitors of Human Neutrophil Elastase

Masateru Miyano,* James R. Deason, Akira Nakao, Michael A. Stealey, Clara I. Villamil, Daniel D. Sohn, and Richard A. Mueller

Department of Immunoinflammatory Diseases Research, Searle Research and Development, 4901 Searle Parkway, Skokie, Illinois 60077. *Received October 7, 1987*

A series of 4-(acyloxy)- and 4,4′-bis(acyloxy)benzophenones were synthesized. Some of them, pivalates (trimethylacetates) and isobutyrates in particular, were found to be potent and selective inhibitors of human neutrophil (leukocyte) elastase. A series of 2-[(acyloxy)methyl]-5-(acyloxy)-4-pyrones were synthesized regioselectively from kojic acid. The 4-pyrones bearing a long chain acyl group at the 2-position and either pivaloyloxy or isobutyryloxy at the 5-position were potent and selective inhibitors of the human elastase. A number of analogues and derivatives in both series were synthesized in order to study the structure–activity relationship as summarized in Tables I–VI and in Tables IX and X. The inhibition was selective to human neutrophil elastase. No inhibition of porcine pancreatic elastase or bovine pancreatic chymotrypsin (Tables VII and XI) was observed. The most likely mechanism of inhibition is discussed. The implication of these findings for the treatment of rheumatoid arthritis and emphysema is outlined.

Elastin is a connective tissue component that provides elasticity to lung connective tissue, yellow tendon, and cartilage of joints. Elastin turnover and remodelling is controlled by neutrophil and tissue elastases. The metabolic turnover of mature elastin is slow under normal circumstances where elastase activity is suppressed by α_1-antitrypsin and α_2-macroglobulin. However, the rate of elastin (and collagen) degradation is greatly enhanced under a variety of clinical conditions,[1] particularly in pulmonary emphysema,[2,3] and rheumatoid arthritis,[4–7]

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